Qualitative and quantitative responses of Diabroticina (Coleoptera: Chrysomelidae) to cucurbit extracts linked to species, sex, weather and deployment method

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Abstract

Toxic baits and traps for Diabroticina, based on bitter cucurbit extracts, have been utilized for a number of years with inconsistent results. Four species of bitter Cucurbitaceae were compared in the field for their attractancy to species of Diabroticina in Argentina and the United States. The comparisons were made with polyester fabrics treated with known volumes of different cucurbit extracts, against a standard cucurbit extract of bitter Hawkesbury watermelon (*Citrullus lanatus* (Thunberg) Matsumura and Nakai). The factors evaluated were: the attractancy of the different extracts in terms of beetle numbers, species and sex of the Diabroticina caught; influence of different fabrics on such attraction; and influence of several weather variables on the catches. The most attractive species was *Cayaponia bonariensis* (Miller) Martinez Crovetto, however, practical considerations indicated that *Cucumis myriocarpus* Naudin and Hawkesbury watermelon may be better choices from the commercial perspective. No single weather factor could explain the catches throughout the sample range, but different temperature and barometric pressure ranges provided some predictive value. Although the susceptibility to weather conditions and a strong male dominance in the catches raise the question of the usefulness of cucurbitacins as the main component in toxic baits or traps, these drawbacks may prove to be less important in widespread bait applications and Diabroticina management in vegetable crops.

Introduction

The subtribe Diabroticina (Coleoptera: Chrysomelidae: Galerucinae) is a large New World group of leaf beetles, of which many are considered pests. The most prominent pests in this subtribe include the corn rootworms and cucumber beetles (*Diabrotica* Chevrolat), cucumber beetles (*Acalymma* Barber) and bean leaf beetles (*Cerotoma* Chevrolat). Regardless of the feeding habits, hosts and geographical distribution of these beetles, most of them have a strong craving for cucurbitacins. Cucurbitacins are oxygenated tetracyclic triterpenoids, usually found combined in glycosides. They are extremely bitter and toxic, serving as chemical defences for plants. However, cucurbitacins have been shown to have strong phagostimulant effects on Diabroticina (Contardi 1939; Howe et al. 1976; Metcalf et al. 1980; Metcalf and Lampman 1989; Nishida and Fukami 1990). Based on these phagostimulant effects, several pest management applications, utilizing cucurbitacins, have been implemented. These pest management applications include traps for monitoring Diabroticina populations (Shaw et al. 1984; Barbercheck et al. 1995; Ventura et al. 1996; Cabrera Walsh 2001; Cabrera Walsh and Cabrera 2004) and formulated toxic baits (Metcalf et al. 1987; Lance and Sutter 1992; Barbercheck et al. 1995).

Bitter cucurbits possess several of the over 20 cucurbitacins and dihydrocucurbitacin types (denoted A, B, C…R) (Rocha Farías and Schenkel 1987). The concentrations and combinations of the different cucurbitacin types vary for different plant species, and between different organs (Metcalf et al. 1982; Lorenzato 1984). Our most common and easily grown sources of cucurbitacins are sources of cucurbitacin E, but cucurbitacin B is detected at lower concentrations by several Diabroticina (Metcalf and Lampman 1989). Raw juices and EtOH : acetone extracts seem to be more attractive than non-polar fractions and purified compounds (Tallamy and Halaweish 1993).

Regardless of the results of laboratory experiments with purified compounds, the field applications of baits and traps, both for commercial and research purposes, rest on formulations based on mother extracts. The form of action of these cucurbit extracts and usefulness in pest management are still controversial because results have often been irregular both for traps and baits. Trap design, and the way the extract in it is prepared, seem to have a great effect on trapping beetles, ranging from a few beetles (<2.6) per day (Shaw et al. 1984; Fielding and Ruesink 1985; Ventura et al. 1996) to hundreds or even thousands of beetles per hour (GCW, unpublished). Likewise, the results of cucurbitacin-based toxic bait applications ranged from no reduction (Barbercheck et al. 1995), to almost complete suppression of beetle populations (Lance and Sutter 1990 Lance and Sutter 1991), with several intermediate situations of moderate to satisfactory control (Chandler 2003; Gerber et al. 2005). In summary, despite the amount of information on the effect and uses of cucurbit extracts, detailed information is still needed on how these extracts work on Diabroticina populations in the field.

In a preliminary work, the extracts of 17 wild cucurbits from Argentina were compared for their attraction power with a standard consisting of a fabric dipped in Hawkesbury watermelon (*Citrullus lanatus* (Thunberg) Matsumura and Nakai) (Hw) juice (Cabrera Walsh 2005). Hawkesbury watermelon was chosen as a standard because it is currently the main source of cucurbitacin (as the E-glycoside) for commercial applications (Schroder et al. 1998, 2001). The original plant list was narrowed down to four extracts dealt with in this work: root extracts of *Cayaponia bonariensis* (Miller) Martinez Crovetto (Cb) and *Cayaponia podantha* Cogniaux (Cp), and fruit extracts of *Cucumis myriocarpus* Naudin (Cm), as well as *C. lanatus* mentioned above. The few full chemical analyses described in the literature indicate that *Cayaponia* species contain B cucurbitacin and their derivatives (Bauer et al. 1985; Rocha Farías and Schenkel 1987; Jacobs and Singh 1990). *Cucumis myriocarpus* Naudin originated in Africa and is adventive in the Americas. There are no available chemical analyses of the fruit of this plant that we are aware of.

Extracts of these cucurbits were studied at the United States Department of Agriculture South American Biological Control Laboratory (USDA-ARS-SABCL), in Argentina, and the USDA-ARS Insect Biocontrol Laboratory (IBL) in Beltsville, MD, USA. Efforts were concentrated on field tests designed to assess the arrestant/attractant strength of the different cucurbit extracts, as affected by beetle sex, weather, extract delivery medium and size of the attractant source, in order to clarify aspects of the cucurbit–Diabroticina interactions. Experiments in different countries are complementary and, because of differences in pest species as well as materials tested, do not constitute replication or quantitatively comparable datasets. These experiments do, according to the objectives, provide comparable qualitative results regarding sexual behaviour towards cucurbitacin-rich extracts, and a ranking of species-specific attraction for the extracts in a wide range of Diabroticina species.

Materials and Methods

Root and fruit extractions

Two different extracts were obtained from the roots and fruit of each plant species. For the first extract, a known weight of root or fruit was milled in an electric cutting mill (Jacobson Pulverator, Jacobson Machine Works Inc., Minneapolis, MN), and allowed to air dry in a shallow tub. The dry sample was immersed for 24 h in EtOH : acetone (3 : 1) at 5–8°C. The sample was then pressed in a printers press inside a fine polyester mesh, so as to separate the liquid from the solids. The solids were suspended again in the same volume of EtOH : acetone for another 24 h. The pressing process was repeated. The solvents were evaporated at 40°C in a shallow tub. The remaining residue was then frozen at −18°C. This was considered the solvent extract. For use in field applications or traps, the extract was suspended in distilled water to the original weight of the sample minus the solid residues.

For the second extract type, the same milling process was conducted on a known weight of root and fruit. The resulting paste was then pressed in a printers press inside a fine polyester mesh, so as to separate the liquid from the coarse solids. The juice was then frozen at −18°C, and thawed at room temperature to allow the remaining starch and fibres to coalesce in lumps that were filtered from the clear liquid phase. The liquid phase obtained was henceforth called raw juice. As this extract was mostly aqueous, it was stored frozen at −18°C to avoid fermentation. In the United States, the Hw juice was fermented, filtered and freeze-dried according to the method of Martin et al. (2002) for later reconstitution with water.

Trap fabrics

The use of white polyester gauze as traps for field collections has been described in previous publications (Cabrera Walsh 2001; Cabrera Walsh and Cabrera 2004). Saturated trap fabrics are made by dipping 0.25 m^2 fabric squares in 20 g (approximately 20 ml) of either fruit or root extracts. Polyester fabrics have limited absorption capacities, so pieces of equal sizes should become saturated with roughly equal volumes of liquid. The fabrics were air dried prior to use. Fifteen other fabrics were tested in the United States using only the standard Hw juice, reconstituted from freeze-dried material (Martin et al. 2002) with deionized water, and normalized to 0.05% content of cucurbitacin E-glycoside using the high pressure liquid chromatography method of Matsuo et al. (1999). The effects of fixed doses vs. saturation of fabrics, as well as the weathering of saturated fabrics, were evaluated in fields in the United States.

Argentine experiments

In Argentina, the trap fabrics were deployed on the ground at the edge of the collection site, upwind from the sampled patch, side by side, separated by roughly 2 m. Simultaneously, the true population densities were estimated through separate manual collections. For this, two operators collected adult beetles with hand-held aspirators throughout the duration of the trapping period, at least 20 m away from the traps. The differences between the proportions of each species represented in the manual and fabric samples was taken to represent the comparative sensitivity of each species to the cucurbitacin-rich extracts. A sample of 100 beetles – or less if unavailable – of the hand-collected beetles was sexed in the laboratory in order to obtain the current sex ratio in the field at the time of sampling.

During these comparisons, the operation of the trap fabrics over 5 and 60 min was monitored in the field. For the 5-min observation, the number of beetles settling on a single cloth extended on the ground was recorded continuously (attractant effect). For the 1-h catch, we recorded the number of beetles collected on the fabrics after that period (arrestant effect). These experiments were designed to determine if the extracts that the beetles found more attractive also kept the beetles on the fabrics for a longer period of time (arrestant effect). During the first collections, a clean piece of polyester fabric was used as an additional control, but this practice was abandoned when it became evident that these fabrics had no attractive properties.

In order to evaluate the attractive power of each plant extract, the proportion of beetles collected on each fabric (beetles on juice × fabric/total beetles on all fabrics) for each sampling date were arcsine transformed, and compared with an ANOVA, followed by Tukey's HSD multiple comparisons. The data used for these analyses were only from the collections of *Diabrotica speciosa* because this species was the most abundant, accounting for 98% of fabric collections, and was the only species present in every South American site.

Wind speed, wind direction, dry bulb temperature, wet bulb temperature, dew point temperature, relative percent humidity and air pressure were registered at the beginning of every sampling period with a hand-held weather gauge (Kestrel 4000, Nielsen Kellerman, Chester, PA). Cloud cover was estimated visually in three categories: clear denotes 0 to 3/10 average sky cover; partly cloudy denotes 4/10 to 7/10 average sky cover; and cloudy denotes 8/10 to 10/10 average sky cover.

Multiple regression analysis and regression tree analysis were applied on the trap data using the weather and density parameters as independent variables (SYSTAT Software, Inc., 2004). Ratios for fabric and manual collections between the eight beetle species captured were compared with 2 × 2 Chi-square tests (SAS Institute 1998), using a Bonferroni correction to experimentwise $\alpha = 0.005$.

United States experiments

Three separate experiments were undertaken in the United States, all conducted at the Beltsville Agricultural Research Station, Beltsville, MD. In all trials, fabric traps, measuring 60 cm × 30 cm, were fixed to a wooden stake. A manila paper folder was attached to the fabric to provide it support. The folder was bent over the top of the stake so as to present a 30 cm × 30 cm vertical surface on both sides of the trap, which was imbedded in the soil such that the lower margin of the fabric was 15 cm above the soil surface, and the two trap surfaces were always facing geographic west and east. The first experiment was conducted in a 0.5-ha field planted to zucchini and summer crookneck squash (*Cucurbita pepo* L.) on 24 and 25 September 2002. This experiment compared 15 different types of fabrics using a measured amount of standard bitter Hw juice. A total of 3.5 ml of juice was applied over the entire surface of fabric (table 1) with a hand-held sprayer. The choice of fabrics was selected based on price and availability. In addition, three of the 15 fabrics (natural muslin, natural burlap and white knitted polyester) were presented as untreated traps (no juice added), or soaked to saturation for 30 min, which resulted in variable quantities of juice absorbed. The volumes of juice absorbed were estimated by utilizing 5 × 5 cm squares in the laboratory, weighed to the nearest 0.01 g before and after saturation, having the excess liquid drained for 30 s before weighing. The mean of four replicate fabric squares was used to estimate the total volume of juice absorbed by the fabric panels when saturated (table 1). In the field, these panels were arrayed in four blocks. Each block was separated by approximately10 m. The panels were embedded between crop rows with 2.5-m separation between traps. The panels were staggered across the row, and rerandomized after each collection.

The second experiment was performed in the same field on 1–7 October 2002, with similar layout as the previous experiment. The same 15 fabrics were soaked to saturation and, in addition, three of the 15 fabrics (natural muslin, natural burlap and white knitted polyester) were presented untreated (no juice added), or as weathered saturated fabric, which had been soaked with juice and then left in the field since the beginning of the first experiment, 24 September. There was significant precipitation during 26 and 27 September, with a total of 38 mm of rain recorded at the weather station located 450 m NNE of the site. No additional precipitation fell during the remainder of the experiments.

A third experiment with treated panels was performed on 7 through 10 October 2003 in adjacent fields, each field measuring approximately 0.3 ha, of seedling zucchini and mature pickling cucumbers (*Cucumis sativus* L.). Two fabrics (yellow felt and white knitted polyester), either untreated or soaked to saturation with standard Hw juice, were presented on panels, as in the previous two experiments, in five blocks in each of the two fields. Blocks were separated by 15 m and traps within blocks were separated by approximately 6 m. Traps were embedded between rows and rerandomized every day after each collection. In the experiment, all *Diabrotica* adults were collected and sexed.

The trials were balanced factorial experiments subject to ANOVA and either means comparison by Tukey's HSD test, pre-planned orthogonal contrasts, or linear regressions using StatView (SAS Institute 1998).

Table 1 Fabrics used for test of panel attraction using bitter Hawkesbury watermelon juice in Beltsville, MD, USA. Measured mass of fabrics and volume of juice absorbed by fabrics when saturated is explained in text

Abbreviation	Panel fabric type	Fibre content (100% unless noted)	Fabric (g/dm^2)	Fabric (g per panel)	Juice (ml per panel)
BURL	Natural burlap	Jute	3.00	54.0	155.9
CARD	Brown cardboard file folder	Paperboard	0.43	7.7	108.3
CHEE	White cheese cloth	Cotton	0.76	13.7	186.8
FELT	White craft felt	Polyester	2.14	38.5	374.3
MUSL	Natural muslin	Cotton	1.17	21.1	53.5
NYLON	White bride crinoline	Nylon	0.57	10.3	11.5
PAMID	White easy-stitch backing	Polyamide	0.67	12.1	13.4
POCO	White trigger poplin	65% Polyester 35% cotton	1.99	35.8	54.2
POLY	White knitted polyester	Polyester	1.30	23.4	94.1
RAYON	White laundered challis	50% Rayon 50% polyester	1.43	25.7	87.9
SATIN	White satin	Acetate	1.12	20.2	26.1
SILK	Dupioni bronze silk	Silk	0.96	17.3	42.6
TOWEL	White paper towel	Paper	2.58	46.4	54.4
WOOL	Grey wool	Wool	2.15	38.7	45.7
YFELT	Yellow craft felt	Polyester	1.83	32.9	390.9

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Results

Cucurbit yields

The *Cayaponia* species grown at the SABCL yielded 8–11 kg of tubers per metre square, equivalent to 5.5–7 kg of raw juice, after 5 years. *Cucumis myriocarpus* produced 7 kg of fruit per metre square, equivalent to 4.5 kg of raw juice, in 3 months.

Trap fabric collections, USA

In Beltsville, all three common Diabroticina were recovered on the trap fabrics: *Diabrotica undecimpunctata howardi* Barber, *Acalymma vittatum* (F.) and low numbers of *Diabrotica virgifera virgifera* LeConte (western corn rootworm).

Trap collection numbers of *D. u. howardi* were compared based on fabric type with measured doses of standard Hw juice (n = 246 beetles total, $F_{14,45} = 5.16$; $P < 0.001$ for day total), as well as for the fabrics saturated with the juice for all collection dates (n = 5232 beetles for all dates, $4.45 < F_{14,45} < 17.11$; $P < 0.001$). A total of 19 *A. vittatum* (striped cucumber beetle) adults were observed over one day at fabric panels with measured juice doses, and 18 adults were detected over 6 days with juice-saturated fabrics, with no detectable effect of fabric type. For *D. u. howardi*, synthetic fabrics treated with measured doses of juice were as a fabric group significantly more attractive than either natural or mixed fabrics (fig. 1) ($F_{2,57} = 20.98$; $P < 0.0001$). Within synthetic and natural fabric groups, no significant differences were detected amongst fabric types. When saturated with juice, there was a weak ($F_{2,57} = 3.33$; $P = 0.043$) tendency for synthetic fabrics to attract higher numbers of *D. u. howardi* than did natural fabrics (fig. 1). However, the variation in *D. u. howardi* numbers on saturated panels was strongly related to the volume each panel was capable of absorbing (fig. 2), which differed greatly between the fabrics (table 1). The regression coefficients did not differ significantly between natural and synthetic fabrics, indicating that when saturated with Hw juice, fabric attractiveness was based on juice content.

Saturated fabrics attracted significantly more beetles of all species than untreated fabrics, and also than the saturated fabrics which weathered 1 week in the field (fig. 3). Saturated panels attracted two times more *D. u. howardi* adults than the panels with measured juice doses, but the differences were not statistically significant. Differences among the three fabrics in the first experiment (fig. 3) were nearly significant for saturated ($F_{2,9} = 4.10$; $P = 0.054$) and significant for dosed panels ($F_{2,9} = 11.79$; $P = 0.0031$). Similarly, differences among the three fabrics in the second experiment (fig. 3) were significant for saturated panels ($F_{2,9} = 4.33$; $P = 0.048$) and panels saturated and weathered ($F_{2,9} = 5.96$; $P = 0.022$). There were no significant differences amongst blank (untreated) fabrics in either experi-

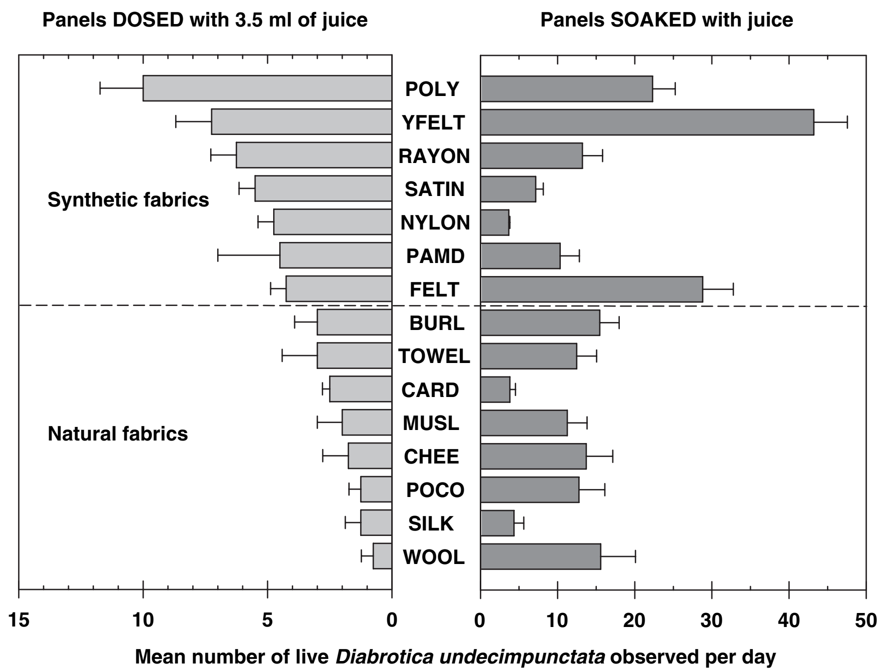

Fig. 1 Comparison of the different fabrics tested as trapping material in terms of the mean number (±SE, n = 4) of *Diabrotica undecimpunctata howardi* observed on a panel, per day, September–October 2002, Beltsville, MD, USA. Each panel was either dosed with 3.5 ml (light bars), or saturated with (dark bars), Hawkesbury watermelon (*Citrullus lanatus*) juice, normalized to 0.05% cucurbitacin E-glycoside. Dosed and shaded bars represent different field tests. For details on fabrics, see Table 1; fabric type ''POCO'' is composed mixed natural and synthetic fibres.

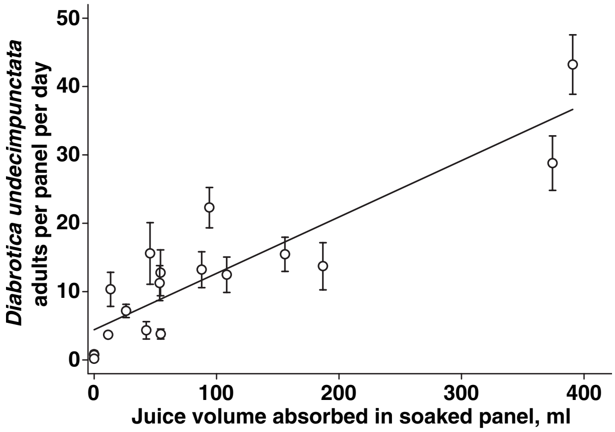

Fig. 2 Regression of mean number (±SE, n = 4) of *Diabrotica undecimpunctata howardi* observed on juice-saturated panels, per day, on estimated juice volume absorbed per panel. Regression line is for all fabric types, including three untreated fabrics; y = 4.42 + 0.0825x; R^2 = 0.792, P < 0.0001. September–October 2002, Beltsville, MD, USA. Each panel was saturated with Hawkesbury watermelon (*Citrullus lanatus*) juice, normalized to 0.05% cucurbitacin E-glycoside. For details on fabrics see Table 2.

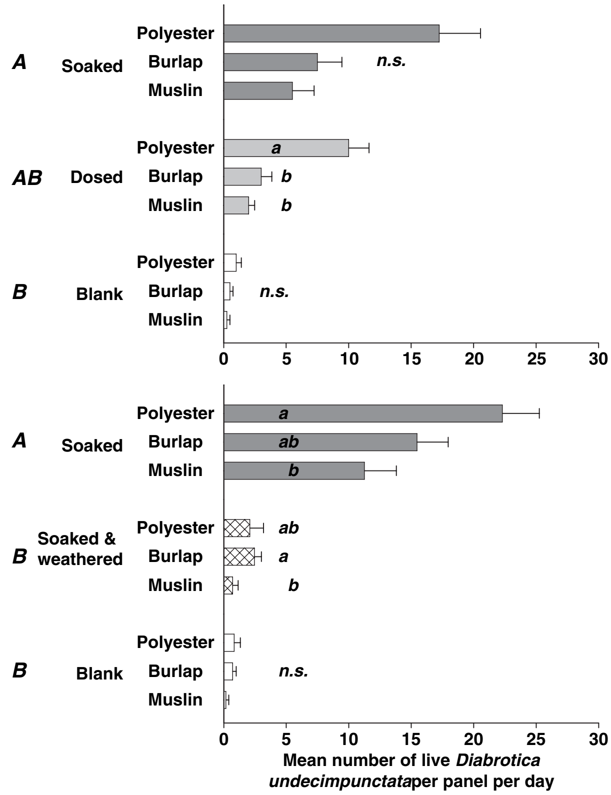

Fig. 3 Comparison of the different fabrics tested as trapping material in terms of the mean number (±SE, n = 4) of *Diabrotica undecimpunctata howardi* observed on a panel, per trap, per day, October 2002, Beltsville, MD, USA. Treated fabrics were either saturated with (dark shaded bars), or dosed with 3.5 ml of (medium shaded bars in top graph only) Hawkesbury watermelon (*Citrullus lanatus*) juice, normalized to 0.05% cucurbitacin E-glycoside, or left untreated (empty bars). Weathered fabrics were soaked and then placed in the field for 1 week (crosshatched bars, lower graph only). Within each graph, upper-case letters indicate significant differences among main treatment effects; lower-case letters indicate significant differences among fabrics within main treatment effects.

ment. The treated POLY fabric was generally the most attractive of the three fabric types.

In the 2003 comparison of POLY and YFELT fabrics saturated in standard Hw juice (fig. 4), contrasts for fabric type were marginal to non-significant ($F_{1,8}$ = 5.57; P = 0.046 for pickles and $F_{1,8}$ = 3.55; P = 0.096 for zucchini). In a comparison between *A. vittatum* collected on saturated fabrics, significantly more beetles were attracted to the YFELT than to the POLY (five times greater; $F_{1,8}$ = 47.9; P = 0.0001).

The sex of *D. u. howardi* on the fabric panels was determined for all adults captured in both fields; 95.7% were males. As only 19 of the over 800 adults were found on the untreated panels (fig. 4), there were insufficient numbers to test the difference in ratios between untreated and juice-saturated panels. *Acalymma vittatum* sexes were not determined.

Trap fabric collections, Argentina

Table 2 shows the species attracted to the fabric traps in Argentina, compared with the hand collections over the same time period, at the same locations and dates. The total number of beetles collected by either method was quite similar. Table 2 ranks the quotient for numbers of beetles attracted to the treated fabrics (beetles on the treated fabrics divided by the beetles obtained in the hand collections), which were classified into three groups. The species *Platybrotica misionensis* Cabrera and Cabrera Walsh, *Diabrotica tripunctata* (F.) and *Diabrotica kirbyi* Baly were highly attracted to the fabrics, although the numbers of *D. kirbyi* were insufficient to distinguish this species from the intermediate group. *Diabrotica speciosa* (Germar), the most abundant and pestiferous Diabroticina in South America, the two *Paranapiacaba* species, and *Diabrotica limitata*, exhibited intermediate attraction quotients. *Diabrotica viridula* (F.), another pest species found primarily on maize (*Zea mays* L.), was significantly less attracted to the fabrics as compared with the other species. A fourth group of Diabroticina, including all South American *Acalymma* (a Diabroticina genus exclusive to cucurbits) and *Cerotoma* spp. (also very common on cucurbits), almost never approached the trap fabrics, although the beetles

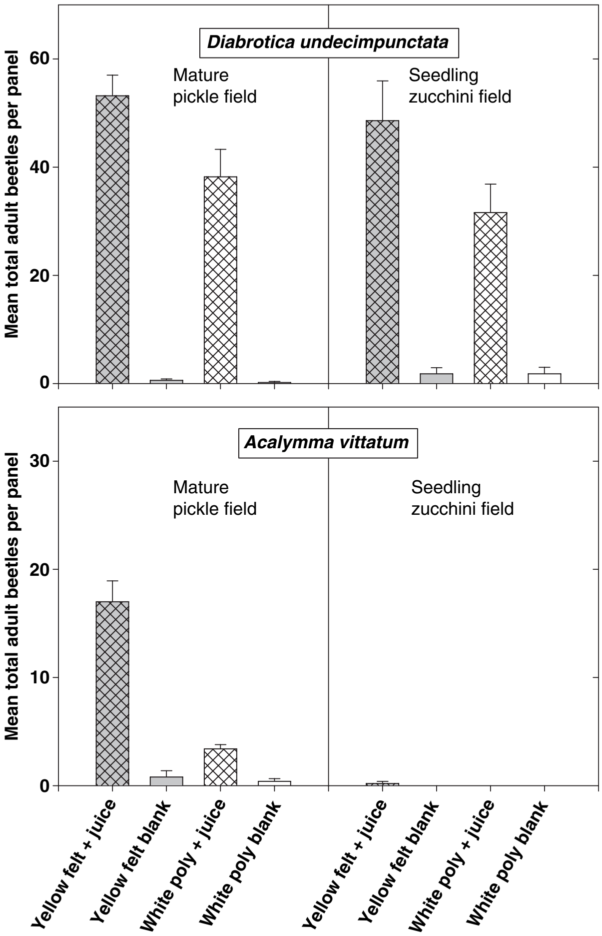

Fig. 4 Comparison of the yellow felt and white knit polyester fabric soaked with juice or left untreated, in terms of the mean total number (±SE, n = 5) of *Diabrotica undecimpunctata howardi* and *Acalymma vittatum* observed per panel, October 2003, Beltsville, MD, USA. Soaked fabrics were saturated with Hawkesbury watermelon (*Citrullus lanatus*) juice, normalized to 0.05% cucurbitacin E-glycoside. Statistical comparisons in text.

were abundant in a number of the sampled vegetation patches (table 2). As in North America, the beetles collected on the treated fabrics were virtually all males (99.85%).

As for the comparative attractiveness of the different juices, table 3 shows the average attractancy of each juice as a proportion of the total capture of the day. Captures on fabrics with *C. bonariensis* were approximately triple of any of the other three extracts in the arresting effect (1-h catch), and around twice in the attractant effect (5-min catch). The ANOVA showed significant differences between the four extracts in both tests (5 min: $F_{3,83} = 5.174$; $P < 0.001$; 1 h: $F_{3,83} = 30.33$; $P < 0.0001$). The

Table 2 Comparative collections of Diabroticina on trap cloths vs. aspirator/sweepnet. The quotient is an index of the relative attraction of each species to the cucurbitacin; quotients followed by a different letter differ significantly by a Bonferroni-corrected 2 × 2 Fisher's exact test at experimentwise $\alpha = 0.05$; overall Chi-square = 817.2 d.f. = 9, $P < 0.0001$

	Trap	Hand	Quotient	
Platybrotica misionensis	113	16	7.06	a
Diabrotica tripunctata	110	20	5.50	a
Diabrotica kirbyi	11	5	2.20	ab
Diabrotica limitata	26	19	1.37	b
Paranapiacaba duodecimmaculata	28	26	1.08	b
Diabrotica speciosa	17 110	16 824	1.02	b
Paranapiacaba significata	15	18	0.83	b
Diabrotica viridula	57	423	0.13	c
Cerotoma arcuata	0	54	0.00	d
Acalymma spp.	0	345	0.00	d
Total	17 470	17 351		

multiple comparisons (Tukey's HSD) indicated that the *C. bonariensis* juice was significantly more attractive than the other juices in both tests, whereas none of the other juices showed significant differences in the attracting and arresting effects.

The average distance at which the observers could spot beetles in flight was around 5 m, and they were observed approaching the traps in direct, unveering trajectories. Once the beetles reached the fabrics, the beetles would land immediately on them, or on the surrounding vegetation. In contrast, beetles were never observed approaching or landing on the untreated fabrics.

The attractive capacity of the treated fabrics did not depend on any single or combination of weather variables (multiple R^2 for the $\log_{10}$ transformed trap catches = 0.286), nor on the hand collection data ($R^2 = 0.021$). A regression tree model was then applied to the data, using the fabric collection figures as the dependent variable. The resulting tree had a fairly good Proportional Reduction in Error (PRE = 0.579), indicating that the splitting predictors can be held partially responsible for the catches differences among sampling dates. These predictors were barometric pressure, which defined a subgroup of low catches (mean = 56.9 beetles/h) when pressure was <1000 hPa; temperatures below 24°C, defining a group of low to medium catches (mean = 129.1 beetles/h); and temperatures below 29°C, discriminating the highest catches (mean = 557.8 beetle/h) from an unaccounted residual group of catches distributed along the frequency plot (table 4; fig. 5).

Table 3 Comparative attractiveness of the four cucurbit juices tested (average of the proportion of *Diabrotica speciosa* captured on each juice per trapping date ± SD)

Cayaponia bonariensis		*Cucumis myriocarpus*		*Citrullus lanatus**		*Cayaponia podantha*	
1 h	5 min	1 h	5 min	1 h	5 min	1 h	5 min
0.57 ± 0.22 n = 25	0.4 ± 0.16 n = 16	0.17 ± 0.09 n = 25	0.21 ± 0.17 n = 16	0.14 ± 0.1 n = 13	0.2 ± 0.05 n = 10	0.19 ± 0.17 n = 25	0.26 ± 0.16 n = 16

*n for this treatment are smaller because standard *C. lanatus* extract was not available on some of the collecting trips.

Table 4 Regression tree analysis table

Node	Count	Mean[a]	SD[a]	Split variable	Cut value	PRE[b]	Improvement	Fit
1	30	205.6	221.4	ATMPRESS	1000	0.17	0.17	0.17
2	8	58.9	41.3					
3	22	259.7	235.9	TEMP	24	0.355	0.186	0.226
4	12	159.7	129.1					
5	10	379.7	283	TEMP	29	0.579	0.223	0.44
6	5	557.8	142.9					
7	5	201.6	283.7					

Predicted variable: CLOTH; fitting method: least squares.
[a]Cloth catches mean and SD of each group defined by the model.
[b]Proportional reduction in error (PRE) = 0.579.

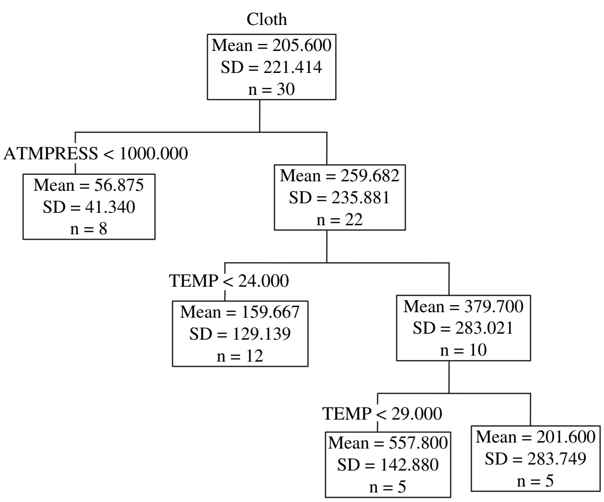

Fig. 5 Regression tree of the trap fabric collection data: weather and manual collection (estimated density) variables were considered the independent variables; whereas the trap fabric collections were considered the dependent variables. Each diagram contains the average, SD and n of the discriminated groups (left side of each node) and residual groups (right side of each node).

Discussion

It is generally accepted that cucurbitacins are, strictly speaking, arrestants, not attractants. This definition implies that leaf beetles only detect these compounds from a short range if they accidentally approach a cucurbitacin source (Metcalf and Lampman 1989; Nishida and Fukami 1990). This definition might in itself contradict the usefulness of cucurbitacins as trap or bait components. Even if this work does not directly challenge this assertion for purified cucurbitacins, the cucurbitacin-rich extracts used in the field can be considered true attractants for four reasons. First, almost no beetles were attracted to the untreated fabrics set close to the treated fabrics. Second, direct trajectories were observed when the beetles approached the saturated and dose measured treated fabrics. Third, the response to different extracts was consistently and significantly different for one of them, indicating that the extract choice at a distance was not random. Finally, the proportional response to increasing juice contents also indicates a quantitative distance response. Commercial baits use raw juices or powdered pulp, not purified compounds, making this study more pertinent to the applied uses of cucurbitacin extracts.

The sensitivity and behaviour towards cucurbitacins is complex among the Diabroticina. This behaviour has been shown to vary among and within the North American species by sex, mating status, prior consumption of cucurbitacins and geographic location (Tallamy and Halaweish 1993; Tallamy et al. 1997; Smyth et al. 2002). Both species of the South American pest *Diabrotica*, *D. speciosa* and *D. viridula*, were attracted to the traps. However, the other important pest Diabroticina species, *Acalymma* spp. and *Cerotoma arcuata*, were not. However, in North America, *A. vitattum* was moderately attracted, indicating that we cannot generalize conclusions to a genus or series level, but need to consider results on a species-by-species basis. Moreover, recorded host plant range for adults was not a good predictor of cucurbitacin attraction.

Different factors must be taken into account to choose suitable plant species for commercial production of cucurbitacin compounds. Although predominance and high concentrations of B cucurbitacins may seem essential because of the elevated attractancy potential, ease of cultivation, abundance and a rapid harvest may be more important factors for plant selection. From this point of view, the most interesting candidates for commercial production of cucurbitacins may be the Hw currently in use, and *C. myriocarpus*. Being annual species, planting and harvesting of the produce would be accomplished within normal crop production time frames, costs and techniques. The productivity per metre square of *C. myriocarpus* (7 kg, GCW, unpublished) was higher than the average productivity per metre square for watermelons (4.5 kg, Florida Agricultural Statistics, 2002). Furthermore, water-soluble compounds such as the cucurbitacin E-glycoside, prevalent in Hw, are subject to disappearance and dilution by rainwater, as shown in our trials with weathered fabric panels in Beltsville. This result may encourage formulators to improve bait water fastness.

Weather, particularly temperature, was a central factor affecting beetle captures, a fact already mentioned by Lance (1990) for field experiments with non-pheromonal volatile attractants. Cool and hot temperatures were not suitable because the beetles were mostly inactive. But, temperatures ranging from 15°C to 34°C were apt for collecting Diabroticina on the trap fabrics. Within this range, however, we could distinguish two groups of catches: low to medium catches with less than 24°C, and the highest catches below 29°C.

Low barometric pressures also separated out a well-defined group of low catches. A number of studies have shown effects of barometric pressure on insect dispersal behaviour and responsiveness to volatile semiochemicals (Lanier and Burns 1978; Lesky and Prokopy 2003, and others). As low, rapidly fluctuating and/or decreasing barometric pressure is associated with stormy weather, several authors have postulated that reduced flight in response to low and/or falling pressure would concentrate flight during calmer, more favourable periods (Lanier and Burns 1978; Fournier et al. 2005, and others).

The highly male-biased attraction for cucurbitacin-treated fabrics across all species of Diabroticina studied is striking but not unexpected. Tallamy et al. (2002) found that male *D. u. howardi* in DE, USA, were disproportionately represented at bitter *Cucurbita andreana* fruits placed in the field, compared with insects sampled >5 m away. Fielding and Ruesink (1985) documented the strong disproportionate dose response of male *D. v. virgifera* and *Diabrotica barberi* Smith and Lawrence to cucurbitacin-containing squash-baited traps. However, most studies have not examined the phenomenon of male bias in response to point sources of cucurbitacin, and its implications for deployment of cucurbitacins in the field.

The strong male dominance in the trap catches raises the question of the usefulness of cucurbitacins as a management tool in toxic baits or traps. Moreover, plant volatiles and either male- or female-produced pheromones (Smyth and Hoffmann 2003 for *Acalymma*; Guss et al. 1983 for *Diabrotica*) may present competition to baits and traps as the beetles colonize host crops. The addition of a different volatile to traps might compensate in part the sexual bias and the competition by other volatiles. In fact, there is evidence that cucurbit pulp contains several attractants, besides cucurbitacins, that attract both sexes (Fielding and Ruesink 1985). In addition, in an experiment comparing several non-pheromonal attractants, Lance (1990) captured similar proportions of each sex in vial traps that used a mixture of carbaryl, olive oil and cucurbitacin to kill the beetles lured by the attractants. However, the design of the trap suggests that the effect of the cucurbitacins themselves in its yield was probably negligible (Shaw et al. 1984; Fielding and Ruesink 1985), and was not evaluated. Toxic cucurbitacin-based baits combined with another non-pheromonal attractant, 1,2,4-trimethoxybenzene + indole + *trans*-cinnamaldehyde (TIC), did not improve the yield of the bait in one experiment (Lance and Sutter 1990), or very mildly so in another (Barbercheck et al. 1995), suggesting it could not significantly influence the sexual proportions. Nevertheless, cucurbitacin-based traps based on our designs could be useful in vegetable agriculture, where the damage is caused principally by adults, regardless of sex, and in monitoring leaf beetle populations.

Optimization of pest monitoring and/or management tactics involving cucurbitacins is a complex challenge, and must test different modes of deployment (such as baits, treated trap crops and toxic traps) with different cucurbitacin-containing extracts (based on cost, efficacy and weathering characteristics), materials (e.g. fabrics, adjuvants) and toxin combinations. Specifically, with respect to the potential of toxic traps and baits in the management of pest Diabroticina, a better understanding will be needed of pest (and non-target organisms) behaviours, including arrival, residence time and departure from treated surfaces, and feeding behaviour while on these surfaces. This will in turn determine the value of cucurbitacins in pest management.

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